Remarks

Upon entry of the Amendment and Reply filed on July 24, 2001, claims 287-622 are pending in the application, with 287, 300, 319, 340, 351, 362, 374, 389, 404, 416, 431, 446, 459, 476, 492, 507, 518, 535, 553, 565, 580, 595, and 608 being the independent claims. The amendments to the specification requested in this Supplemental Amendment correct typographical errors, update the specification to correspond to the sheets of formal drawings submitted on July 24, 2000, and update references to certain provisional applications in the background section. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Reconsideration of this application and entry of the above Amendments are respectfully requested.

Respectfully submitted,

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Date: September 24, 2001

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Version with markings to show changes made

Please amend the paragraph on Page 4, lines 3-9, as follows:

Several unique receptors for TRAIL have already been identified. In [co-pending] U.S. provisional patent application no. 60/035,722, DR4, a novel death domain containing receptor for TRAIL, was disclosed. See, Pan *et al.*, *Science 276*:111-113 (April, 1997). The TR5 receptor, the subject of [co-pending] U.S. provisional patent application 60/035,496, has now been shown to bind TRAIL. In co-pending U.S. provisional patent application no. 60/[xxxxxx]050,936, it was predicted that the TR10 receptor would also bind TRAIL, owing to sequence homology with DR4.

Please amend the paragraph on page 4, lines 18-21, as follows:

The present invention provides for isolated nucleic acid molecules comprising nucleic acid sequences encoding the amino acid sequence shown in FIG. 1A and 1B (SEQ ID NO:2) or the amino acid sequence encoded by the cDNA clone deposited as ATCC Deposit No. 97920 on March 7, 1997.

Please further amend the first four paragraphs on page 6, lines 3-33 (as previously amended on October 26, 1999), as follows:

FIG. 1A and 1B show[s] the nucleotide (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of DR5. It is predicted that amino acids 1-51 (underlined) constitute the signal peptide (amino acid residues from about -51 to about -1 in SEQ ID NO:2); amino acids 52-184 constitute the extracellular domain (amino acid residues from about 1 to about 133 in SEQ ID NO:2); amino acids 185-208 (underlined) constitute the transmembrane domain (amino acid residues from about 134 to about 157 in SEQ ID NO:2); and amino acids 209-411 constitute the intracellular domain (amino acid residues from about 158 to about 360 in SEQ ID NO:2), of which amino acids 324-391 (italicized) constitute the death domain (amino acid residues from about 273 to about 340 in SEQ ID NO:2).

FIG. 2A, 2B, and 2C show[s] the regions of similarity between the amino acid sequences of DR5 (HLYBX88), human tumor necrosis factor receptor 1 (h TNFR1) (SEQ ID NO:3), human Fas protein (SEQ ID NO:4), and the death domain containing receptor 3 (SEQ ID NO:5). The comparison was created with the Megalign program which is contained in the DNA Star suite of programs, using the Clustal method.

FIG. 3 shows an analysis of the DR5 amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graph, amino acid residues about 62 to about 110, about 119 to about 164, about 224 to about 271, and about 275 to about 370 as depicted in [Figure 1] FIG.

1A and 1B (SEQ ID NO:2) correspond to the shown highly antigenic regions of the DR5 protein. These highly antigenic fragments in [Figure 1] FIG. 1A and 1B (SEQ ID NO:2) correspond to the following fragments, respectively, in SEQ ID NO:2: amino acid residues from about 11 to about 59, from about 68 to about 113, from about 173 to about 220, and from about 224 to about 319.

FIG. 4 shows the nucleotide sequences (HAPBU13R (SEQ ID NO:6) and HSBBU76R (SEQ ID NO:7)) of two cDNA molecules which are related to the nucleotide sequence shown in [Figure 1] FIG. 1A and 1B (SEQ ID NO:1).

Please further amend the paragraph on page 7, lines 20-29 (as previously amended on October 26, 1999), as follows:

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a DR5 polypeptide having the amino acid sequence shown in FIG. 1A and 1B (SEQ ID NO:2), or a fragment of the polypeptide. The DR5 polypeptide of the present invention shares sequence homology with other known death domain containing receptors of the TNFR family including human TNFR- I, DR3 and Fas (FIG. 2A, 2B, and 2C). The nucleotide sequence shown in FIG. 1A and 1B (SEQ ID NO:l) was obtained by sequencing cDNA clones such as HLYBX88, which was deposited on March 7, 1997 at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, and given Accession Number 97920. The deposited clone is contained in the pSport 1 plasmid (Life Technologies, Gaithersburg, MD).

Please amend the paragraph on page 8, lines 20-29, as follows:

The determined nucleotide sequence of the DR5 cDNA of SEQ ID NO:1 contains an open reading frame encoding a protein of about 411 amino acid residues whose initiation codon is at position 130-132 of the nucleotide sequence shown in FIG. 1A and 1B (SEQ ID NO.1), with a leader sequence of about 51 amino acid residues. Of known members of the TNF receptor family, the DR5 polypeptide of the invention shares the greatest degree of homology with human TNFRI, FAS and DR3 polypeptides shown in [Fig. 2] FIG. 2A, 2B, and 2C, including significant sequence homology over multiple cysteine-rich domains. The homology DR5 shows to other death domain containing receptors strongly indicates that DR5 is also a death domain containing receptor with the ability to induce apoptosis. DR5 has also now been shown to bind TRAIL.

Please amend the paragraph on page 9, lines 5-17, as follows:

Therefore, the present invention provides a nucleotide sequence encoding the mature DR5 polypeptide having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit No. 97920, and as shown in [Figure 1] FIG. 1A and 1B (SEQ ID NO:2). By the mature DR5 protein having the amino acid sequence encoded by the cDNA clones contained in the host identified as ATCC Deposit

No. 97920, is meant the mature form(s) of the DR5 protein produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the vector in the deposited host. As indicated below, the mature DR5 having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97920, may or may not differ from the predicted "mature" DR5 protein shown in SEQ ID NO:2 (amino acids from about 1 to about 360) depending on the accuracy of the predicted cleavage site based on computer analysis.

Please further amend the paragraph on page 9, line 25-page 10, line 2 (as previously amended on October 26, 1999), as follows:

In the present case, the predicted amino acid sequence of the complete DR5 polypeptide of the present invention was analyzed by a computer program ("PSORT"). See, K. Nakai and M. Kanchisa, *Genomics 14*:897-911 (1992). PSORT is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis by the PSORT program predicted the cleavage sites between amino acids 51 and 52 in [Figure 1] FIG. 1A and 1B (-1 and 1 in SEQ ID NO:2). Thereafter, the complete amino acid sequences were further analyzed by visual inspection, applying a simple form of the (-1,-3) rule of von Heinje. von Heinje, *supra*. Thus, the leader sequence for the DR5 protein is predicted to consist of amino acid residues from about 1 to about 51, underlined in [Figure 1] FIG. 1A and 1B (corresponding to about -51 to about -1 in SEQ ID NO:2), while the predicted mature DR5 protein consists of residues from about 52 to about 411 in [Figure 1] FIG. 1A and 1B (corresponding to about 1 to about 360 in SEQ ID NO:2).

Please amend the paragraph on page 11, lines 15-27, as follows:

Preferred nucleic acid fragments of the present invention include, but are not limited to nucleic acid molecules encoding: a polypeptide comprising the DR5 extracellular domain (amino acid residues from about 52 to about 184 in FIG. 1A and 1B (from about 1 to about 133 in SEQ ID NO:2)); a polypeptide comprising the DR5 transmembrane domain (amino acid residues from about 185 to about 208 in FIG. 1A and 1B (from about 134 to about 157 in SEQ ID NO:2)); a polypeptide comprising the DR5 intracellular domain (amino acid residues from about 209 to about 411 in FIG. 1A and 1B (from about 158 to about 360 in SEQ ID NO:2)); and a polypeptide comprising the DR5 death domain (amino acid residues from about 324 to about 391 in FIG. 1A and 1B (from about 273 to about 340 in SEQ ID NO:2)). Since the location of these domains have been predicted by computer graphics, one of ordinary skill would appreciate that the amino acid residues constituting these domains may vary slightly (e.g., by about 1 to 15 residues) depending on the criteria used to define each domain.

Please amend the paragraph on page 11, line 34 to page 12, line 10, as follows:

Preferred nucleic acid fragments of the present invention further include nucleic acid molecules encoding epitope-bearing portions of the DR5 protein. In particular, such nucleic acid fragments of the present invention include, but are not limited to, nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 62 to about 110 in [Figure 1] FIG. 1A and 1B (about 11 to about 59 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about 119 to about 164 in [Figure 1] FIG. 1A and 1B (about 68 to about 113 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about 224 to about 271 in [Figure 1] FIG. 1A and 1B (about 173 to about 220 in SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 275 to about 370 in [Figure 1] FIG. 1A and 1B (about 224 to about 319 in SEQ ID NO:2). The inventors have determined that the above polypeptide fragments are antigenic regions of the DR5 protein. Methods for determining other such epitope-bearing portions of the DR5 protein are described in detail below.

Please amend the paragraph on page 13, lines 3-9, as follows:

Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of the DR5 cDNA shown in [Figure 1] FIG. 1A and 1B (SEQ ID NO:1)), or to a complementary stretch of T (or U) resides, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

Please amend the paragraph on page 14, line 34 through page 15, line 9, as follows:

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a DR5 polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the DR5 polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The reference (query) sequence may be the entire DR5 nucleotide sequence shown in FIG. 1A and 1B (SEQ ID NO:1) or any polynucleotide fragment as described herein.

Please amend the paragraph on page 27, lines 6-17, as follows:

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in [Figure 1] FIG. 1A and 1B (SEQ ID NO:2), the amino acid sequence encoded by deposited cDNA clones, or fragments thereof, can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

Please amend the paragraph on page 28, line 27 through page 29, line 4, as follows:

The present inventors have discovered that the DR5 polypeptide is a 411 residue protein exhibiting three main structural domains. First, the ligand binding domain was identified within residues from about 52 to about 184 in FIG. 1A and 1B (amino acid residues from about 1 to about 133 in SEQ ID NO:2). Second, the transmembrane domain was identified within residues from about 185 to about 208 in FIG. 1A and 1B (amino acid residues from about 134 to about 157 in SEQ ID NO:2). Third, the intracellular domain was identified within residues from about 209 to about 411 in FIG. 1A and 1B (amino acid residues from about 158 to about 360 in SEQ ID NO:2). Importantly, the intracellular domain includes a death domain at residues from about 324 to about 391 (amino acid residues from about 273 to about 340 in SEQ ID NO:2). Further preferred fragments of the polypeptide shown in FIG. 1A and 1B include the mature protein from residues about 52 to about 411 (amino acid residues from about 1 to about 360 in SEQ ID NO:2), and soluble polypeptides comprising all or part of the extracellular and intracellular domains but lacking the transmembrane domain.

Please amend the paragraph on page 29, line 35 through page 30, line 8, as follows:

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate DR5-specific antibodies include: a polypeptide comprising amino acid residues from about 62 to about 110 in [Figure 1] FIG. 1A and 1B (about 11 to about 59 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about 119 to about 164 in [Figure 1] FIG. 1A and 1B (about 68 to about 113 in SEQ ID NO:2); a polypeptide comprising amino acid residues from about 224 to about 271 in [Figure 1] FIG. 1A and 1B (about 173 to about 220 in SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 275 to about 370 in [Figure 1] FIG. 1A and 1B (about 224 to about

319 in SEQ ID NO:2). As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the DR5 protein.

Please amend the paragraph on page 36, lines 12-29, as follows:

Potential antagonists [acccording] according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, *Science* 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy DR5 mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, *Nature* 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of DR5 (FIG. 1A and 1B). [Preferrably] Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the DR5 mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. Since ribozymes, unlike antisense molecules are catalytic, a lower intracellular concentration is required for efficiency.

Please amend the paragraph on page 49, lines 13-16, as follows:

The 3' primer for DR5 has the sequence 5'-CGCGGTACCTTAGCCT GATTCTTTGTGGAC-3' (SEQ ID NO:12) containing the underlined Asp718 restriction followed by nucleotides complementary to the DR5 nucleotide sequence in FIG. 1A and 1B, followed by the stop codon.